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The Role of Cognition in Sexual Signals and Mate Choice Decisions

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The Role of Cognition in Sexual Signals and Mate Choice Decisions

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ABSTRACT

Cognitive ability varies dramatically among individuals, yet the manner in which this variation affects reproduction has rarely been investigated. Here, we asked 1) whether male sexual signals reflect cognitive ability and whether females prefer males with superior cognitive abilities, and 2) whether female cognition affects male and female mating decisions? We addressed these questions in a mutual mate choice system, threespine sticklebacks (*Gasterosteus aculeatus*). We tested cognitive performance by presenting the males and females with a novel task (a barrier to food) to evaluate problem-solving abilities and learning. We found that males that problem solve have elaborate sexual signals and are preferred by females. However, contrary to our expectations, female cognitive abilities did not influence male courtship vigor or their own mating decisions. In dynamic environments, such as the rivers where these fish live, problem-solving ability is a neurological attribute that may preferred by females because it is important in the contexts of male foraging or parental care. Additionally, we argue that males and females experience different selection pressures for cognitive abilities, and therefore express different preferences for cognition in their mates. Our research adds to the expanding body of literature linking cognition and sexual selection, demonstrates that cognitive ability may play an important role in a diverse array of mating systems, and explains how cognition may assist in the maintenance of variation sexual signals within natural populations.

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INTRODUCTION

Most animal interactions involve communication. Males attract females with showy ornaments, offspring beg for food, toxic prey warn their predators with bright colors, and competitive interactions are mediated through scents, physical attributes, and behavior. In all of these interactions, communication occurs between a sender and a receiver, and the signals are shaped by selection. Signals can be broadly defined as acts or structures that alter the behavior of another organism (Davies et al. 2012). Thus, one ubiquitous aspect of animal communication is that signals are effective because the receiver's response has evolved to detect and assess the transmitted signal. Additionally, communication can be quite elaborate, involving several sequential steps, occurring in multiple modalities, and integrating behaviors and signals in a synchronized fashion. Communication is also situational, involving learned behaviors or dynamic responses that can depend on seasonality, environmental variables, and personalities of the interacting organisms, for example. This complexity means that signals can be both demanding to display and problematic to decipher. As a result, researchers have considered the role that cognition has to play in animal communication. Cognition can be broadly thought of as the neurological method by which organisms obtain, process, recall, and apply information that they have acquired about their environments (Shettleworth 2001; Dukas 2004).

The research described in this thesis focuses on communication between the sexes, assessing the role of cognition in signaling for mates and the behavioral responses to those signals that occur during mate choice. Signals and displays used to express and examine the quality of potential mates are typically called 'sexual signals'. In most systems, females are the choosy sex, while males compete for mates (as a result of the imbalance in energy invested in gametes; Trivers 1972). Research on which male traits (such as coloration, behavior, song, and pheromones) are targets of female choice and why females have preferences for these particular traits has flourished in recent decades (Andersson 1994). Males often exhibit these traits to females in a courtship display. Like most animal communication, sexual signals involve elaborate displays. For males to be chosen as mates, they must coordinate these courtship signals to create an effective display. Furthermore, females must perceive and assess these elaborate displays, in order to select a mate with high fitness. Recently, researchers have uncovered a role for male cognition in elaborate male displays; males signal cognitive ability through sexual displays and females express preferences for male cognitive ability (detailed in Chapters 1 and 2; Boogert et al. 2008; Keagy et al. 2009, 2011). Additionally, authors have suggested that female cognition might play a role in their ability to make mate choice decisions (Keagy et al. 2009, 2012). This connection, however, remained untested until now (Chapter 2).

Cognitive ability may be selected by females because of the benefits provided in aspects of an animal's life as divergent as foraging and predator avoidance (Dukas 1998). Additionally, cognitive ability confers a benefit by buffering individuals from

environmental stressors, thereby lowering mortality (Sol et al. 2007). Although possessing superior cognitive abilities appears to aid animals in survival-related components of fitness, the role of cognition in reproduction is seldom considered (Keagy et al. 2009; Boogert et al. 2011). By mating with individuals equipped with enhanced cognitive abilities, females may directly and/or indirectly benefit. Higher cognition males may provide additional resources such as food and/or superb territories (direct benefits) or contribute 'good genes' to future offspring (indirect benefits) (Keagy et al. 2009; Boogert et al. 2011).

Thus far, all research assessing cognition and mating decisions has considered the impact of male cognition on male signals and female preferences. Male cognition is correlated with particular sexual signals, including song repertoire complexity in zebra finches (Boogert et al. 2008), wing-bar size in siskin (Mateos-Gonzalez et al. 2011), and behavioral displays in satin bowerbirds (Keagy et al. 2012). Moreover, females prefer males with superior cognitive abilities such as maze learning in guppies (Shohet and Watt 2009), and problem-solving in bowerbirds (Keagy et al. 2009). Links between both cognition and sexual signals, and cognition and mate choice have only been established in one organism, bowerbirds (Keagy et al. 2009, 2011, 2012). It is critical to examine this relationship in a variety of different species. If male cognition is important for mating decisions in divergent species with dramatically different mating systems, it may play a more important role in sexual selection universally.

Here, I compare male and female cognitive abilities and determine how cognition affects mating decisions in threespine sticklebacks (*Gasterosteus aculeatus*). I chose

threespine sticklebacks as a study system because they differ from bowerbirds (the only other system in which connections between sexual signals and cognition and female choice and cognition have been made) in important ways. With regard to their reproductive biology, male sticklebacks provide all parental care (Wootton 1976), while female bowerbirds provide all the parental care (Vellenga 1980). Furthermore, most threespine sticklebacks are annual fish, whereas bowerbirds are long-lived and spend several years preparing for their first opportunity to mate (Vellenga 1970). These differences in life history are likely to impact whether cognition plays a role in sexual selection and what aspects of cognition are sexually selected. In addition to being very different from bowerbirds, sticklebacks are an ideal system for examining cognition and mate choice for two reasons. First, they have elaborate, well-characterized courtship displays. Females assess male color signals (Milinski and Bakker 1990; Scott 2004; Flamarique et al. 2013), courtship behaviors (Rowland 1995), body size and shape (Nagel and Schluter 1998; Head et al. 2013), and nest characteristics (location: Candolin and Voigt 1998; concealment: Sargent 1982; decoration: Ostlund-Nilsson and Homlund 2003). Males may benefit from high cognitive abilities if it helps them to coordinate these elaborate displays, while high cognitive performance may be useful for females to properly evaluate male signals. Second, since males make a large reproductive investment, providing all the parental care in this system (Wootton 1976), they exhibit mate choice preferences (Johnstone et al. 1996). This allows me to ask important unanswered questions about whether males express preferences for female cognitive

ability. Moreover, if cognition impacts parental care ability, there may be additional selective pressures for females to choose a male with superior cognitive abilities.

In this thesis, I address how both male *and* female cognitive abilities impact mate choice using threespine sticklebacks as a model system. I use a novel foraging task (presented repeatedly) to assess two measures of cognitive ability, problem solving and learning via operant conditioning. In the first chapter I ask 1) do threespine stickleback sexual signals indicate male cognitive ability and 2) do females choose males based on male cognitive abilities? Briefly, for the first time, I demonstrate relationships between cognition, sexual signals, and mate choice in a non-avian model system. Male sticklebacks expressing a preferred color signal were more likely to solve the novel foraging task on the first presentation. Moreover, male problem solving ability is important in female choice. Females were more likely to enter the nests of males who innovated and solved a novel foraging task in few attempts on the first presentation. In the second chapter, I ask 1) do males and females differ in cognitive ability 2) do males prefer females that perform well on cognitive tasks and 3) do differences in female cognition impact female mate choice decisions? In short, I found dramatic differences in cognitive ability on a novel foraging task between the sexes. This likely reflects variation in the selection pressures to which the two sexes are exposed. However, I found no evidence for male preferences for females who performed well on my cognitive task, and no evidence that female cognition impacts their own mating decisions.

My research adds to a growing body of literature that links cognition and communication between the sexes during mate choice and tests, for the first time, the role

of female cognition in sexual selection. My results suggest that cognitive abilities may play an important role in animal communication in many diverse systems. I discuss my results in the contexts of sex differences in cognition, how diversity in sexual signals are maintained both within and between populations, and the degree to which preferred cognitive traits parallel sex-specific cognitive challenges.

CHAPTER ONE: COGNITION IS INDICATED BY ATTRACTIVE SEXUAL SIGNALS AND PROBLEM-SOLVING MALES PREFERRED BY FEMALES IN MATE CHOICE

Introduction

Animal courtship displays can be strikingly elaborate. They may contain several sequential steps, occur in multiple modalities (e.g. visual, acoustic, and tactile signals), and involve the integration of signals and behavior. For example, peacock spiders synchronously use motion displays, body ornamentation, and vibrations in their courtship display (Girard et al. 2011). Proper coordination of these courtship signals is essential for them to operate as an effective display. Recent findings suggest that cognitive ability may underlie both elaborate displays and female assessment of complex male signals (Boogert et al. 2008; Keagy et al. 2009; Ryan et al. 2009; Boogert et al. 2011).

Cognition is broadly defined as the neurological manner in which animals acquire, process, retain, and apply information they obtain about their surroundings (Shettleworth 2001; Dukas 2004). Cognition aids in vital elements of survival such as foraging and predator avoidance (Dukas 1998) and can buffer animals from environmental stressors, lowering mortality (Sol et al. 2007). Yet, cognition is rarely studied in mating contexts (Keagy et al. 2009; Boogert et al. 2011). Evidence is growing that cognition may assist males in obtaining mates. For instance, male bowerbirds adapt their courtship behaviors to avoid startling females, which increase male reproductive success (Patricelli et al 2002, 2006). Furthermore, superior foragers may signal their foraging ability to females via

exaggerated sexual signals in carotenoid-dependent signaling systems (Endler 1980). Additionally, females may also prefer mates with particular enhanced cognitive abilities that are situationally advantageous because of the direct and/or indirect benefits females obtain when mating with them (Keagy et al. 2009; Boogert et al. 2011).

Although in its infancy, the study of the role of cognition in sexual selection has begun to develop as a field (see review in Boogert et al. 2011). Sexual signals such as song complexity have been linked to novel foraging tasks in zebra finches (Boogert et al. 2008), suggesting that foraging ability may be communicated through sexual signals. Furthermore, female guppies prefer males who learn mazes quickly (Shohet and Watt 2009). Male guppies use carotenoid based signals, and those males who learn the maze quickly (potentially indicating foraging abilities) may produce higher quality signals (Karino et al. 2007). However, the link between both sexual signals and cognition, and female preferences and cognition, has been made in only one system, satin bowerbirds. Male satin bowerbird mating success is related positively to their cognitive performance and overall problem-solving ability (Keagy et al. 2009; Keagy et al. 2011). Females appear to select high performing mates by assessing several behavioral displays, which indicate male cognitive ability (Keagy et al. 2012). Here, we demonstrate for the first time relationships between cognition, sexual signals, and mate choice in a non-avian model system, threespine stickleback (*Gasterosteus aculeatus*). In addition to being deeply divergent taxa, stickleback life history differs dramatically from bowerbirds. For instance, most sticklebacks live 1-2 years and exhibit paternal care, while bowerbirds are long-lived (reaching maturity after 7 years) and provide maternal care. Moreover, male

sticklebacks provide both direct and indirect benefits to offspring, whereas bowerbirds provide only indirect benefits. These key life history differences may impact whether cognition is tightly linked to sexual selection and what aspects of cognition are sexually selected. If cognition is an important aspect of stickleback sexual selection, as it is in bowerbirds, we may expect cognition is an important characteristic many mating systems. Alternatively, if cognition does not play a role in this system, cognition may only be important during mating interactions in particular taxa.

Threespine sticklebacks are excellent candidates for examining the role of cognition in mate choice for two main reasons. First, male sticklebacks provide all parental care. Thus, along with contributing gametes, males who perform well on challenging cognitive tasks may be better able to provide direct benefits for eggs and fry. This could increase the incentive for choosing a 'high cognition' mate, if cognition is related to parental care ability. Second, stickleback sexual signals and courtship are elaborate, and preferences are well established in the literature. Male courtship behavior occurs in a stepwise fashion (Nagel and Schluter 1998) and females prefer males who vigorously court (von Hippel 1996). Males court females with a courtship "dances" that help display body coloration, an additional signal (Bell and Foster 1994). Males produce carotenoid-based red color on their throats and iridescent blue eyes during the breeding season. Females have strong preferences for extensive and intense red throat coloration (Ter Pelkwijk and Tinbergen 1937; Milinski and Bakker 1990; Scott 2004; Tinghitella et al. 2015) and blue eye color (Rowland 1994). Recently it has been suggested that the contrast between the iridescent blue eye and the red throat may be the most preferred

signal (Flamarique et al. 2013). Nest quality and placement also appear important in some populations; males that select safe (Candolin and Voigt 1998), concealed nesting locations (Sargent 1982), and decorate them properly (Östlund-Nilsson and Homlund 2003) are preferred by females. Furthermore, male courtship occurs in multiple modalities: visual (Milinski and Bakker 1990, Flamarique et al. 2013), tactile (Nagel and Schluter 1998) and olfactory (McLennan 2003; Kozak et al. 2011). If paternal care is cognitively taxing, females may prefer males with superior cognitive abilities. Males may use elaborate courtship (flashy color signals, behavioral sequences with multiple steps and modalities, and/ or integrating of both behaviors and signals) to display their cognitive abilities to females.

Here, we consider three questions 1) do threespine stickleback sexual signals indicate male cognitive ability 2) does cognitive ability predict time to build a nest, and 3) do females choose males based on male cognitive abilities? We consider speed of nest building and female choice to be measures of male fitness. We assessed cognition using a novel barrier test with a food reward, which was presented to males several times over a period of seven days. Our design assesses the cognitive aspects of problem-solving and learning via operant conditioning (behavior controlled by reinforcement or punishment, also called 'trial and error learning'; Thorndike 1911; Skinner 1938; Staddon and Cerutti 2003) as measures of cognition. Our performance measures were whether individuals solved the barrier test, attempts taken per "solve", and time to solve. Each of these measures was assessed on each day of presentation. We then measured sexual signals, time spent nest-building, and female acceptance of males. Our hypotheses were that 1)

male sexual signals are reflective of cognitive ability, 2) cognitive abilities predict time spent building a nest, and 3) females express preferences for male cognitive ability. We made the following predictions. First, we anticipated males with higher quality sexual signals, such as larger redder throats and intense blue eyes would complete the barrier test more often, in fewer attempts, and in less time. Second, we expected males that perform better on our cognitive task to construct their nests more quickly. Finally, we expected females to choose high preforming males as mates, preferring to interact with those males who performed better (solving the barrier, learned quickly etc.) on the barrier test.

Methods

We collected reproductive threespine sticklebacks from the Chehalis River (46° 58' 42"N, 123° 28' 46"W) in SW Washington, USA, in April 2014. We transported fish to the University of Denver and housed them in single sex groups in 110-L (77 x 32 x 48 cm) or 284-L (123 x 47 x 54 cm) home tanks at a density of approximately one fish per 5-L. We fed all individuals in home tanks a mixture of brine shrimp (*Artemia* sp) and bloodworms (chironomid larvae) and just brine shrimp on alternating days. We kept fish in a temperature and photoperiod controlled room set to 17°C and 15:9 h light:dark cycle in the beginning of the experiment (May, 2014). Broad-spectrum (400-900nm) Sylvania Octron Eco 5000K fluorescent lights illuminated the room. We adjusted the lights and temperature throughout the breeding season to track conditions in SW Washington. Before trials began, we relocated individuals from their home tanks to randomly assigned visually isolated 100-L (77 x 32 x 48 cm) experimental tanks. We conducted cognition

testing and mate choice in these tanks. Each tank contained an artificial plant, gravel pack (crushed gravel in a nylon casing), and a nesting container (17 long x 11 wide x 3 tall cm) filled with sand and covered by half of a flower pot (15 long x 16 base x 7 tall cm). We provided 5g of nesting material (*Ceratophyllum demursum*) to each male. These objects mimicked natural environments and allowed for males to build nests.

Barrier Test

To measure male cognitive ability we designed a novel barrier test with a food reward. This test measured the cognitive processes of problem-solving and operant conditioning (also called 'trial and error learning'; Thorndike 1911; Skinner 1938; Staddon and Cerutti 2003).

Before beginning the barrier test, we allowed 24 hours (days 0-1) for fish to acclimate. During this 24-hour period we did not feed experimental fish, since food was used as an incentive to solve. We conducted the first barrier test 24 hours after introduction to the testing tank (day 1). Before each trial began, we lowered an opaque divider into the tank, blocking the fish's view of the barrier apparatus and food reward as they were placed into the tank. We used a transparent plastic container (11.5 cm diameter base, 7 cm tall) with a 9.5cm diameter opening on the top as our barrier (Figure 1). In the center of the clear plastic container, we suspended a small clear plastic bag (3 x 2.5cm) containing bloodworms and water. We used three bloodworms attached to the outside of the bag via petroleum jelly as the food reward. We began observations when the opaque divider was removed. To retrieve the food reward, the fish needed to swim above and

into the cylinder through the 9.5cm diameter opening. The trial concluded when the fish entered the barrier or after 10 minutes.

We recorded whether the fish solved the test (entered the barrier), the number of attempts each fish made to access the food (as evidenced by physically contacting the barrier), as well as the time to solve the barrier test (from the start of the trial to entering the barrier) using the event recorder JWatcher (Blumstein et al. 2006). We removed from our analyses all fish that did not attempt to solve the barrier test in any of the four trials. Individuals that solved without physically contacting the barrier (1 solve/ 0 attempts) were given a score of 1 (the maximum score for solves/attempts). We assigned the maximum possible solving time (600 seconds = 10 minutes) to all non-solvers. We fed non-solvers three bloodworms to standardize food obtained across individuals. In experimental tanks, aside from the three bloodworms provided during each cognition trial, we only fed fish brine shrimp as a daily source of food. Feeding not associated with the barrier test, always occurred temporally (at least one hour) removed from the cognition trial. Once the trial ended, we removed the barrier and food reward bag. We conducted subsequent barrier trials on days two, four, and seven, always following a 24-hour break from food. We compared performance on the barrier test across days, which allowed us to test learning through operant conditioning. We used a learning curve to assess performance over the subsequent trials (Skinner 1938). Our approach to measuring cognition by incorporating multiple presentations of a problem-solving task has recently been advocated for in literature (Thornton et al. 2014). However, we are aware that measures of cognition that do not have direct parallels in the animals' natural habitats

have been criticized recently (Rowe and Healy 2014). Although the specific task of maneuvering through a clear plastic cup would not be naturally encountered, animals encounter novel foraging situations in which they must navigate obstacles and problem solve to obtain food. Following the fourth barrier test, we weighed all males to the nearest hundredth of a gram using a digital balance (Scout Pro SP202) and returned them to their home tanks. A total of 58 males were presented with barrier tests.

Nesting

To prompt males to begin constructing and continue maintaining nests we introduced randomly chosen gravid females into experimental tanks daily (enticement) for 10 minutes. On days on which the barrier test was conducted, this enticement period always occurred after the male's cognition trial was completed. If a male had completed nest building, enticement took place with a female enclosed in a jar to prevent spawning. We considered a nest to be "under construction" if the male had begun to fasten down sand or plant materials with a glue-like protein used in nest building. We considered nests complete when an opening and exit were clearly visible (Wootton 1976). Males that did not begin to nest during the seven days over which cognition was measured were given a second opportunity to nest after all males had completed cognition trials. Additionally, we removed all males that did not completely construct a nest by day 14. The maximum number of days an individual could spend in an experimental tank was 28 (14 days for the first opportunity and 14 for the second opportunity), therefore we assigned all individuals that did not nest a score of 28. Male sticklebacks that construct nests quickly may have higher fitness because they will be able to secure mates before others. For

instance, females often prefer males that arrive first to mating sites (Møller 1994; Aebischer et al. 1996; Lozano et al. 1996; Velmala et al. 2015).

Mate Choice

To assess female choice, we conducted no-choice mating trials with methods commonly used by multiple labs (Nagel and Schluter 1998; Head et al. 2009; Tinghitella et al. 2013). After a male had completed nest building, a gravid female was placed into a releasing device (an opaque cylinder with a manually operated exit) within a nesting males' tank. After a 2-minute acclimation period, the female was released into the tank and we recorded 18 behaviors related to mate choice (Appendix 1) in real time, again using JWatcher, for the duration of the 20-minute trial or until the female entered the nest. We used each male in mate choice trials up to two times; two males were only used once because they did not maintain their nests long enough to be paired with a second gravid female. Males underwent their second mate choice trial soon after the first (1.42 ± 0.22 days) and were enticed on the days on which mate choice was not conducted. We used females up to two times with the exception of two females (one who was used three times, the other four). We never paired males and females with the same mate. Similar to pervious work, we allowed males and females to rest for at least two hours between mating trials (Kozak et al. 2013; Tinghitella et al. 2015). To determine body condition of individuals that participated in mate choice trials, we calculated the residuals from a regression of weight and length (Jakob et al. 1996). Following each trial, we photographed males and females under standardized conditions to determine the length component of body condition (Taylor et al. 2006; Cooper et al. 2011). We obtained a

measure of length for each fish by using TPSDIG2 (Rohlf 2006a,b). We placed a landmark on the anterior and posterior extents of the body and multiplied length by a scale factor (obtained from each photo) corresponding to 1 mm. We massed females to the nearest hundredth of a gram (Scout Pro SP202) and tagged them with an individualized elastomer mark (Northwest Marine Technology Inc) so that we could identify individuals after they were released back into their home tanks. We tagged all males after cognition and mate choice trials were completed. We completed 52 courtship trials with 27 males and 38 females.

Sexual Signals

We measured male color signals including the throat, eye, and body color. We scored male color on a scale of 0-5 with half point increments by eye using standardized methods (Boughman 2001, 2007; Lewandowski and Boughman 2008; Lackey and Boughman 2013; Tinghitella et al. 2013; Tinghitella et al. 2015) that reliably match reflectance data (Albert et al. 2007; Boughman 2007). We additively combined (equally weighted) throat area and intensity into one measure that we call "throat score" (Lackey and Boughman 2013). We measured color in all males after at least two hours removed from contact with females. Additionally, we scored males who received mate choice trials both before and after each trial, because male sexual signals changed considerably during mate choice trials (personal observation). We used the average of the pre- and post trial color scores when including color in models assessing female preference because this is a better reflection of what females observed. In addition to examining male coloration, we measured nest area and courtship vigor. Larger nests may indicate

readiness to invest energy towards reproduction (McKaye et al. 1990; Soler et al. 1998; Östlund-Nilsson and Homlund 2003). To measure nest area, we photographed nests using a Canon Powershot G15 equipped with a Canon WP-D48 waterproof case. We measured area in nest photos using ImageJ version 1.47 (http://rsb.info.nih.gov/ij/). We outlined the perimeter of the nest and established scale using a ruler visible in each photo. To quantify male vigor, we summed all male courtship behaviors directed towards the female and divided by trial duration (behaviors/minute).

Statistical Analysis

To assess learning on our repeated barrier tests, we measured how male performance (solving the barrier test, solves per attempts, and time to solve) changed over the four trials using Friedman nonparametric tests. We used three separate models (one with each cognitive measure as a response) and trial number (1, 2, 3, 4) as the predictor. We performed these analyses in R (Core Team, 2013, version 2.1.2) using 'friedman.test' function in the 'stats' library.

To determine whether males' sexual signals indicate cognitive ability we used generalized linear models. We examined solving the barrier, solves per attempts, and time to solve as our response variables. When necessary, we transformed our response variables to improve normality. We arcsine square root transformed solves per attempts, and log transformed time to solve. For each response variable we were interested in how individuals performed when the task was novel (problem-solving; trial one) and how they learned. To obtain learning scores, we performed regressions on scores over the four trials for each individual fish, and used the slope from these regressions as the learning

score. For example, if a fish solved in fewer attempts on each sequential trial, the learning score would be positive. For each response variable, we ran two models (trial one and learning). The measures of color-based sexual signals were throat color score (area + intensity), eye intensity, body intensity, and their interactions. We dropped non-significant interactions from models in all of our analyses. We used male mass as a covariate in all models assessing the relationship between sexual signals and cognition. We performed these analyses using JMP version 11.0.

To assess if other (non-color) sexual signals, such as courtship behavior and nest characteristics, are correlated to cognitive performance we used the subset (27 individuals) of males that built nests and underwent mate choice trials. Only males that built nests and had courtship trials could be measured in these categories. First, to determine if nest size is predicted by cognition we looked at the three cognition performance measures (solving the barrier, solves per attempts, and time to solve) for trial one and learning separately in models that had nest area as the fixed effect. Next, to determine if courtship vigor is predicted by cognition we examined the three cognition performance measures for day one and learning with courtship vigor (number of male courtship behaviors per second) as the fixed effect. We averaged nest area and courtship vigor over each male's trials, because we were concerned with the male's signal and not his response to different females. We performed these analyses using JMP version 11.0.

To establish whether males' cognitive abilities predict time spent building a nest we used generalized linear models. We used time building (days to a complete nest after introduction to the experimental tank) as our response variable. We looked at solving the

barrier, solves per attempts, and time to solve for trial one and learning in separate models as fixed effects. We performed these analyses using JMP version 11.0.

Finally, we used generalized linear mixed models to address whether females preferred males with higher cognitive ability. The binomial response variable was whether or not the female entered the nest (Yes/No) for spawning. In separate models, we used solving the barrier, solves per attempts, and time to solve for trial one and learning as our fixed effects. Because some individuals were used more than once, male and female IDs were random effects. Color signals (throat color score, eye intensity, and body intensity) and male body condition were covariates. We included coloration and body condition in the models to determine if females preferred cognition regardless of color signals and condition signals. We compared each model (with one main effect) to a reduced null model that included only random effects, color signals, body condition, and random effects (no fixed effects) and determined statistical significance of the fixed effects using chi-squared tests (Winter 2013). We performed these analyses in R (R Core Team, 2013, version 2.1.2) using the 'glmer' function in the 'lme4' library.

Results

Barrier Test and Cognition

We found that solving frequency and time to solve changed over the four trials. Frequency of solving increased over trials (Friedman test, $F_r = 22.24$, $df = 3$, $p < 0.001$; Appendix 2a). Additionally, time to solve decreased over the four trials (Friedman test, $F_r = 35.86$, $df = 3$, $p < 0.001$; Appendix 2b), while solves per attempts did not change over the four trials (Friedman test, $F_r = 4.02$, $df = 3$, $p = 0.26$; Appendix 2c).

Sexual Signals and Cognition

Color signals, but not courtship vigor or nest area, predicted male cognitive ability. Males who solved the barrier test on day one had an eye intensity that was more than twice that of non-solvers (Table 1). We also found significant interactions between eye score and throat score and between body score and throat score on solving on day one (Table 1, Figures 2a, b). These interactions indicate that males with more contrast between eyes and throat and between body and throat were more likely to solve on day one (Figures 2a, b). Higher throat score was not, on its own, a predictor of problem-solving or learning (Table 1). Solving in fewer attempts and solving in less time on day one were not related to color signals, however body mass predicted how quickly individuals solved on day 1 (Table 1). The only sexual signal measured that was associated with our measures of learning (regression of trials 1-4) was eye intensity, which was negatively correlated with the slope of males' learning curves indicating that males with duller eye color were more likely to solve on later days (2, 4, and/or 7). Color signals were not associated with learning for solves per attempts or time to solve (Table 1). Moreover, neither nest size nor courtship vigor were predictors of any cognition measures (solving, solves per attempts, or time to solve) for day one or learning (Tables 2, 3).

Nest Building and Cognition

Time spent constructing a nest was not predicted by our cognitive measures. Contrary to our prediction, males with superior problem-solving and/or learning abilities did not build nests more quickly (Table 4).

Female Preference and Cognition

We found evidence for a significant relationship between female mate choice and male cognition. Namely, the males who were accepted by females as mates (as evidenced by females entering their nests for spawning) were more likely to solve the barrier test and to solve it in fewer attempts on the first presentation (Table 5). Accepted males were 30% more likely to have solved on day one than males who were not accepted (Table 5, Figure 3a). Moreover, males that were not accepted by females made almost three times the number of attempts on day one as accepted males (Table 5, Figure 3b). In some stickleback populations, acceptance of a male (entering their nests) is rare, and thus female choice is assessed with other measures (Head et al. 2009; Kozak et al. 2009). In our study, females entered nests in 54% of trials, making entering a nest a useful metric of mate acceptance in this population. Female sticklebacks are known to prefer males with exaggerated color signals (Milinski and Bakker 1990; Flamarique et al. 2013), however, we found that measures of cognition predicted female acceptance of males even in models that also contained color signals. We found no association between female acceptance and time to solve on day one (Table 5). In addition, we found no connection to female preference and learning for any of the three measures (Table 5).

Discussion

Our results demonstrate the first link between cognition, sexual signals and female preferences in a non-avian system. We found that sexual signals convey information about male cognitive abilities. Males that solved the barrier on day one had more intense blue eyes and females from some stickleback populations prefer intense

blue eyes (Rowland 1994). Furthermore, day one solvers had greater contrast between eye and throat, and between body and throat color scores, suggesting that contrast between conspicuous color signals is related to cognitive abilities (Table 1, Figure 2a, b). This contrast is an important signal. Recent research has demonstrated that blue eye signals pre-dated red throat signals in sticklebacks and the red throat may have developed to produce a stronger contrast with the blue eye (Flamarique et al. 2013). Rearing environment such as nutrient stress or enrichment has large implications for brain development (Kempermann et al. 1997, Nowicki et al. 1998). Intense blue eyes may be used to convey cognition since rearing environment also has implications for the production of structural pigments (Hill 2006). Our findings show that color signals are one way cognition is communicated to potential mates.

Eye intensity was negatively associated with the learning curve for solving over the four trials (Table 1). This is likely due to solving being a yes or no response (whereas other measures are continuous). Because of this, males that solve on day one cannot improve (which would be indicated by a positive slope for our learning curve) over the three other trials. Therefore, a day one solving males' learning curve could either be completely flat (solved the barrier on all four trials) or be negative (if they did not solve on one or more of the subsequent days). This is unlike our other two cognitive measures, in which problem-solving males could solve in fewer attempts or less time during subsequent trials.

We expected males that performed well on our cognitive task to build nests in less time, however we found no evidence of this link (Table 4). High performing males could

potentially locate nesting materials quickly and construct a nest with fewer errors. However, in a lab setting, search time for suitable nesting materials is likely shorter than in natural habitats, and therefore potentially not as cognitively taxing. Additionally, building time in natural habits would likely depend on other factors such as when the male arrived on the breeding grounds, territory establishment, and defense against competing males. Conversely, cognition may not play a role in nest construction. We think this is unlikely since males build similar nests when given multiple opportunities to construct a nest (Rushbrook et al. 2008).

Our measures of courtship behavior (vigor) and nest characteristics (area) did not predict cognitive ability (Tables 2, 3). However, females did select high performing males, even when color signals were included in our models. Therefore, females may assess male cognition through courtship behavior and/or nest characteristics not tested in this study. For example, one potentially promising area of courtship behavior is plasticity (Patricelli et al 2002, 2006). Males may display cognitive abilities by better adapting courtship displays in response to female receptiveness (Keagy et al. 2009). Females may also prefer males who adapt their courtship behavior to female interest. Experiments using "Any Fish" (software that allows researchers to create a computer-animated courting fish; Veen et al 2013) would offer an excellent opportunity to test this idea, since female or male behavior could be controlled. Additionally, in this study we only measured nest area; however, females pay attention to a host of other nest characteristics (location: Candolin and Voigt 1998; concealment: Sargent 1982; decoration: Östlund-Nilsson and Homlund 2003). Future studies could provide variation in nest site

concealment and materials, to determine if these elements vary among males that differ in cognitive ability. Furthermore, female cognition could play an important role in mate choice decisions (Keagy et al. 2009; Keagy et al. 2012), affecting the ability of females to identify male quality in systems with elaborate and multi-modal courtship systems like sticklebacks. Females who themselves perform poorly on tests of cognition may be less choosy, while 'high cognitive' females may be picky. Another possibility is that females mate assortatively with respect to cognitive ability (Boogert et al. 2011).

We predicted that females would prefer males who performed well on cognition tests. We found that females were more likely to accept (enter the nest) males who solved or solved in fewer attempts on day one, regardless of color signals (Table 5). Although females preferred high quality color signals (as described in earlier literature; Milinski and Bakker 1990; Flamarique et al. 2013), these results suggest females may select high performing males by assessing cognition displayed through other means (e.g. courtship behavior, nest characteristics, etc.). Female preference for cognitive abilities communicated through non-color related traits may be an additional explanation for why there is so much variation in sexually selected traits like color in natural populations, despite strong directional selection for exaggerated signals.

When investigating relationships between cognition and reproduction, the aspects of cognition that are particularly important to females will likely depend on the environment experienced by an organism (Boogert et al. 2011). Innovation allows animals to exploit novel resources (Reader and Laland 2003). Problem-solving tasks have been suggested as an appropriate tool for examining innovation (Griffin and Guez 2014).

Therefore, problem-solving abilities may be important in dynamic environments in which individuals experience constant change (Tebbich et al. 2010). The Chehalis River has a rapid water flow and meters of daily tidal fluctuations (personal observation). The expression of and preference for problem-solving tendencies could have evolved in response to these changing environments. Innovators in other systems tend to be low ranking individuals (Reader and Laland 2003; Thornton and Samson 2012), but these innovators inhabit terrestrial environments which are typically less dynamic. Evidence from spatial learning research in sticklebacks suggests environment (lake vs river) impacts solving strategy (Girvan and Braithwaite 2000; Braithwaite and Girvan 2003). Therefore, an interesting next step would be to compare female preference for problem-solving in lake residing sticklebacks (a less dynamic environment) to that in populations from dynamic rivers. We do not expect females from lake populations to show strong preferences for males that problem-solve, since lakes are more stable environments. In this study, male performance on the first presentation of the barrier test was associated with both male color signals and female acceptance. Color signals predicted whether the male solved the barrier test on day one, while both solving and solves per attempts predicted female choice. At least in this system, learning abilities (in terms of operant conditioning) do not seem to be closely associated with sexual signals or mating decisions (Table 5).

In summary, color signals, but not courtship vigor or nest area, are predictive of cognitive ability (problem-solving) and females prefer males that problem solve. The preference for problem solvers parallels the dynamic environments experienced by this

population of river-dwelling fish. Cognition could be in linkage disequilibrium with male sexual signals and/or female preferences. Furthermore, in many populations there is strong directional selection favoring exaggerated male traits. However, there is still a great amount of within-population variation in sexual signals. Preference for cognitive abilities may be an additional explanation for this variation in signals. Animal cognition appears to play an important role in vastly divergent mating systems, suggesting it may play a more integral role in sexual selection than previously thought.

CHAPTER TWO: COGNITION IS NOT ALWAYS PREFERRED IN MATE CHOICE: THE ROLE OF FEMALE COGNITION IN MALE AND FEMALE MATING DECISIONS

Introduction

Identifying traits preferred in mate choice and why they are preferred has been a focus of sexual selection research for the last few decades. Typically, this research has focused on female preferences for male traits like coloration, behavior, song, and pheromones (Andersson 1994). Recently, male cognition has been identified as a trait preferred by females in several divergent species (bowerbirds: Keagy 2009; guppies: Shohet and Watt 2009; sticklebacks: (Chapter 1)). Cognitive ability might be preferred by females because of the impacts it has on an individual's fitness (Sol et al. 2007). The role of female cognition in problem-solving and other tasks unrelated to mating has been identified as a potentially important factor in mating decisions (Keagy et al. 2009; Keagy et al. 2012) however, this has yet to be assessed. Female cognition may play two distinct roles during mate choice. First, cognition impacts fitness (Sol et al. 2007), so in systems where males provide more than just gametes and are themselves choosy, males may express preferences for females with superior cognitive abilities. Second, because courtship displays can be remarkably elaborate (occurring in multiple modalities, containing sequential steps, and involving the coordination of signals and behavior) and females must perceive, integrate, and assess these complex displays, female mating decisions may themselves be dependent on cognitive ability (Keagy et al. 2009; Keagy et

al. 2012). In this paper, we ask whether males choose females that perform well on a problem-solving and learning task, and whether their own cognitive performance impacts their choice of mates.

The role of cognition in mate choice is a relatively new area of research, and until now, no study has assessed male and female mating decisions based on female cognition using an extractive foraging task. Male cognition is associated with male sexual signals including song repertoire complexity in zebra finches (Boogert et al. 2008), color signals in sticklebacks (Chapter 1), and multiple behavioral displays in bowerbirds (Keagy et al. 2012). Additionally, females prefer males who perform well on certain cognitive tasks including maze-learning in guppies (Shohet and Watt 2009) and problem-solving in bowerbirds and sticklebacks (Keagy et al. 2009; Chapter 1). Clearly, aspects of male cognition impact sexual signaling and female preferences.

Although female cognitive performance on non-mate choice tasks has not been assessed in this context, differences in male and female cognitive abilities have been examined a number of times. For instance, depending on the study, male or female primates are more likely to innovate (male: Reader and Laland 2001; female: Kawai 1965), and, in fish, female guppies are more likely to innovate (Laland and Reader 1999). Yet, in other systems, innovative tendencies do not differ between the sexes (Benson-Amram and Holekamp 2012). Additionally, males and females also differ in other cognitive abilities, for instance, male voles (Gaulin and FitzGerald 1986, 1989) and rats (Seymoure et al. 1996) show enhanced spatial learning, while female guppies (Reader and Laland 2000) and chimpanzees (Lonsdorf et al. 2004) have superior social learning

abilities. These sex differences in innovation, spatial learning, and social learning are likely products of selection molding males and females in response to sex-specific challenges, such as navigating in a large home range (Gaulin and FitzGerald 1986) or asymmetric prioritization of food resources (Reader and Laland 2000). Thus, we compare male and female cognitive abilities in this paper to understand how potential similarities or differences may play a role in mate selection. Males may prefer different aspects of female cognition or not express preferences for female cognitive abilities. Moreover, high performing females may be able to recognize high quality mates with fewer errors than low performing females. Additionally, if male and female cognitive abilities differ (like in other systems), and sex specific preferences for cognitive abilities parallel cognitive performance, we might gain additional insight into the evolution of mate preferences for cognitive abilities.

We chose threespine sticklebacks (*Gasterosteus aculeatus*) as our model system to ask if female performance on a cognitive task impacts mate choice for three main reasons. First, we recently demonstrated that male cognition plays a role in mating decisions in female sticklebacks. Our previous work showed that female sticklebacks from Southwest Washington rivers prefer males that problem-solve as mates (Chapter 1). Second, male sticklebacks make a large investment in offspring, and male mate choice is well-established in this system (Rowland 1982, 1989; Sargent et al. 1986; Kraak and Bakker 1998). Male sticklebacks defend a territory, build nests, and provide all parental care for eggs and juveniles (Wootton 1976), so we should expect them to exhibit mate choice preferences (Johnstone et al. 1996). We can thus ask whether males express

preferences related to female cognitive ability, and if so, whether those preferences differ from female preferences for male cognition. Finally, male sticklebacks have well-characterized, elaborate courtship displays. Females assess male color signals (Milinski and Bakker 1990; Scott 2004; Flamarique et al. 2013), body size and shape (Nagel and Schluter 1998; Head et al. 2013), courtship behaviors (Rowland 1995), and nest characteristics (location: Candolin and Voigt 1998; concealment: Sargent 1982; decoration: Ostlund-Nilsson and Homlund 2003). Because female sticklebacks evaluate many male signals, superior cognitive abilities may be advantageous in processing and comparing these elaborate sexual signals.

Here, we considered three questions: 1) do males and females differ in cognitive performance, 2) do males prefer females that perform better on cognitive tasks, and 3) does female cognition impact female mate choice decisions? We examined cognition using a barrier test with food as a reward (Chapter 1). We presented this test multiple times to each individual tested, allowing us to measure both problem-solving and learning via operant conditioning (also called 'trial and error learning'; Thorndike 1911; Skinner 1938; Staddon and Cerutti 2003). Our measures of performance on the barrier test were whether individuals solved the barrier test, attempts taken per "solve", and time to solve. We made several predictions. First, we expected males to be more likely to solve the barrier test, and to solve the test in fewer attempts and less time than females on the first presentation. Male sticklebacks typically forage solitarily while defending nesting territories, and females forage in shoals (Hart and Gill 1994); male sticklebacks may therefore be more likely to rely on problem-solving in foraging contexts.

Additionally, at least in some populations, male sticklebacks have much larger brains than females. This male size difference is the largest known in any invertebrate (Kotrschal et al. 2012). In populations where males do not provide parental care this brain size dimorphism is reversed (Samuk et al. 2014). Brain size is also associated with superior cognitive skills in some systems (Reader and Laland 2002). For similar reasons (foraging behavior, brain size differences) we expect males to perform better on learning aspects of the barrier test, improving more dramatically over the four trials. With regard to female cognition and male mate choice, we expected males to more vigorously court females who performed well on the barrier test because those females are likely to possess genes for high performance on cognitive tasks that will be passed onto offspring. Although males examine female body condition as a proxy of fecundity, the same trait could also be used as a metric for female cognitive ability, since females with superior cognitive abilities may be better foragers and thus be in better condition. Finally, we expected females that perform better on the barrier test to be better able to assess complex sexual signals, and thus be more selective. High-performing females should discriminate more strongly against males with less-preferred sexual signals and/or lower cognitively performing males. In contrast, females who perform less well on the barrier test shouldn't be able to distinguish high quality mates as readily.

Methods

We collected threespine sticklebacks from the Chehalis River (46° 58' 42"N, 123° 28' 46"W) in SW Washington, USA, in April 2014 using minnow traps. We separated the fish by sex before transporting them to the University of Denver where they were housed

in 110-L (77 x 32 x 48 cm) and 284-L (123 x 47 x 54 cm) holding tanks at a density that did not exceed one fish per 5-L. We set the lab temperature and photoperiod to 17°C and a 15:9 h light:dark cycle in the beginning of the experiment and adjusted the light:dark cycle throughout the breeding season to replicate conditions in SW Washington. We fed all individuals a mixture of brine shrimp (*Artemia* sp) and bloodworms (chironomid larvae) and only brine shrimp, on alternating days. At the start of cognitive testing individuals were relocated from their holding tanks to randomly assigned, visually isolated, 110-L (77 x 32 x 48 cm) experimental tanks. We allowed males to build nests in these experimental tanks during the seven days over which cognition trials occurred (see below). These tanks contained a green plastic plant, a gravel pack, a nesting tray (17 x 11 x 3 cm) filled with sand, half a flower pot (15 x 9 x 7 cm) covering the nesting tray, and 5g of live plant material (*Ceratophyllum demursum*) used for nest building. These items mimicked natural settings and allowed males to construct nests.

Barrier Test

We used a barrier test to measure male and female cognition. The results of male performance on the barrier test were presented in Chapter 1, and are included here in order to make comparisons between male and female cognition. The barrier was a transparent plastic container (11.5 cm diameter base, 7 cm tall) with a 9.5cm diameter opening on the top (Figure 1). In the center of the clear plastic container, we suspended a small clear plastic bag (3 x 2.5cm) containing bloodworms and water. The reward for solving the barrier test was three bloodworms attached to the outside of the bag via petroleum jelly. We allowed fish to acclimate to their experimental tanks for 24 hours

(days 0-1) before testing. During this acclimation period, we deprived the fish of food, since food was used as an incentive to solve. Immediately before each trial, we lowered an opaque divider into the tank to block the fish's view while the barrier and reward were placed into the tank. The trial began when we removed the opaque divider. To solve the barrier and obtain the food reward, the fish needed to swim above and into the plastic container through the 9.5 cm diameter hole. We observed each trial for 10 minutes, or until the fish solved the barrier test.

We recorded three aspects of fish behavior including solving (entering the barrier), number of attempts (as evidenced by physically contacting the barrier), and time to solve (from the beginning of the trial) in real-time using the event recorder JWatcher (Blumstein et al. 2006). We conducted subsequent trials on days two, four, and seven to measure learning. Fish were always tested after 24-hours without food. We removed all fish that did not make an attempt to solve the barrier on any of the four trials from our analyses. We gave fish that solved without making an attempt (1 solve/ 0 attempts) a score of 1 (the maximum score for solves/attempts). Fish that did not solve the barrier were assigned the maximal length of time (600 seconds = 10 minutes). To standardize food received, we fed non-solvers three bloodworms at least an hour after trials were conducted. To supplement their diet while undergoing cognition trials, we also fed the fish brine shrimp daily. After the final cognition trial, we massed all fish to the nearest hundredth of a gram using a digital balance (Scout Pro SP202). A total of 58 males and 55 females completed barrier tests.

Mate Choice

We allowed males to construct nests during the 7-day course of cognition trials. To promote nest construction, we released a randomly chosen gravid female, who was currently not undergoing cognition trials, into each male's tank for 10 minutes daily. On days that we conducted barrier testing, this enticement always occurred after all cognition trials for that day were completed. After seven days, if a male had not begun nest construction we removed him from the experimental tank. If, on day seven, a male had begun constructing a nest, we allowed an additional seven days (14 total) for the male to complete the construction. We considered a nest to be "under construction" if the male had begun to fasten down sand or plant materials with a glue-like protein used in nest building. We considered nests complete when an opening and exit were clearly visible (Wootton 1976). We removed any male that began to construct, but did not completed a nest on day 14. Once all males completed cognition trials ("first chance"), any males that did not build a nest during their "first chance" were given a "second chance" to build a nest and go through mating trials in a subsequent week.

We assessed male courtship vigor and female choice by conducting no-choice mating trials using methods commonly used by multiple labs (Nagel and Schluter 1998; Head et al. 2009; Tinghitella et al. 2013). We used males and females in mate choice trials up to two times with two different mates, with the exception of two females (one who was used three times, the other four). Similar to previous work, we allowed at least two hours between mating trials (Kozak et al. 2013; Tinghitella et al. 2015). We color scored males before and after each trial to capture how sexual signal expression varied

throughout the trial, since color can change quite rapidly (personal observation). We measured male color signals including the throat, eye, and body color by eye using standardized methods (Boughman 2001, 2007; Lewandowski and Boughman 2008; Lackey and Boughman 2013; Tinghitella et al. 2013; Tinghitella et al. 2015) that reliably match reflectance data (Albert et al. 2007; Boughman 2007). We combined throat area and intensity additively into one measure that we called "throat score" (Lackey and Boughman 2013). We took the average of pre- and post trial color scores to produce the color scores used in statistical models that assessed female choice.

For each courtship trial, we first acclimated females to the male's tank in an opaque releasing device for two minutes before they were freed into the tank. We then recorded all behaviors associated with mate choice (Table S1) in real time, again using JWatcher for 20 minutes or until the female entered the nest. We carefully removed females from the nest before they could deposit their eggs. Following courtship trials, we photographed each fish under standardized conditions and massed females to the nearest tenth of a gram (Scout Pro SP202). We obtained a measure of length for each fish using TPSDIG2 by placing a landmark on the anterior and posterior extent of the body and multiplying the resulting length by the scalar obtained from each photo (Rohlf 2006a,b). Finally, we determined body condition of each individual by calculating the residuals from a regression of body mass and length (Jakob et al. 1996). We tagged all fish with an individualized elastomer mark (Northwest Marine Technology Inc) to identify individuals after they were released back into their home tanks. We completed 52 mate choice trials with 27 males and 38 females.

Statistical Analyses

To determine how male and female performance changed over the four trials for each of our cognitive measures (solving the barrier test, solves per attempts, and time to solve) we used Friedman nonparametric tests. We ran three separate models for each sex (one with each cognitive measure). In each of these models, one cognitive measure was our response variable and trial number (1, 2, 3, 4) was the predictor. These analyses were performed in R (Core Team, 2013, version 2.1.2) using the 'friedman.test' function in the 'stats' library.

To address whether males and females differ in cognitive performance we used generalized linear models. In these models, cognitive measure (solving the barrier, solves per attempts, or time to solve) was the response, and sex was the fixed effect. These analyses were performed in JMP version 11.0.

To address male interest in females with varied cognitive abilities we used linear mixed models. Male courtship vigor (number of male courtship behaviors per minute, excluding nest related behaviors, see Table S1) was our response variable. We examined three cognitive measures as fixed effects: solving the barrier, solves per attempt, and time to solve. For each of the three fixed effects we were interested in how individuals performed when the barrier was novel (trial 1) and whether performance improved over time (learning across trials 1-4), so we ran two models for each response variable (one representing problem-solving and one representing learning). To obtain our learning scores, we first calculated a regression of performance over the four trials for each individual. The slope from these regressions was the learning score. For example, if an

individual solved in fewer attempts on each sequential trial, the learning curve would be positive. When necessary, we transformed our fixed effects to improve normality. We arcsine square root transformed solves per attempts, and log transformed time to solve. We included female body condition as a covariate because males preferably court more fecund females in some populations (Rowland 1982, 1989; Sargent et al. 1986; Kraak and Bakker 1998). Because some individuals were used more than once, male and female IDs were random effects. We compared each model (with one fixed effect) to a reduced null model that included only female body condition and random effects (no fixed effects). We determined statistical significance of the fixed effects using chi-squared tests (Winter 2013). These analyses were performed in R (R Core Team, 2013, version 2.1.2) using the 'lmer' function in the 'lme4' library.

To address if female body condition indicates female cognitive performance we used generalized linear models. In these models, female condition was the response, and cognitive measure (solving the barrier, solves per attempts, or time to solve) was the fixed effect. These analyses were performed in JMP version 11.0.

To address whether female cognition affects female mating decisions we used generalized linear mixed models. Our response variable was whether or not the female entered the nest (Yes/No) for spawning. A significant interaction between female cognition and male sexual signals on mate acceptance would indicate that females that performed differently on the barrier test also differed in their acceptance of male mates. Thus, in each model we included one female cognition measure, a male sexual signal (color or cognition), and the interaction of the two. We investigated three female

cognitive abilities on two time scales as our main effects (a total of six main effects): solving the barrier, solves per attempts, and time to solve for problem-solving (trial one) *and* for learning (trials 1-4). The male sexual signal main effects were throat score and eye score, and the male cognition measures were solving the barrier and solves per attempts on day one. Throat and eye color signals were used because females express strong preferences for these traits (Milinski and Bakker 1990; Scott 2004; Flamarique et al. 2013). Solving and solves per attempts on day one were used because we recently demonstrated that females are more likely to accept males who possess these cognitive abilities as mates (Chapter 1). We additionally included male and female IDs as random effects and male body condition as a covariate because in some populations of sticklebacks females prefer males that are in better condition (Bakker et al. 1999). Because we were specifically interested in the interaction between female cognition and male sexual signals, we compared each model including one interaction to a reduced null model that included the fixed effects (same pair, only without the interaction), male body condition, and random effects. We determined statistical significance of the interaction effects using chi-squared tests (Winter 2013). We corrected for multiple comparisons by using a Bonferroni adjustment. These analyses were performed in R (R Core Team, 2013, version 2.1.2) using the 'glmer' function in the 'lme4' library.

Results

Barrier Test and Cognition

Males and females both showed improvement in cognitive performance over time. Females' frequency of solving (Friedman test, $F_r = 20.30$, $df = 3$, $p < 0.001$; Figure 4) and

solves per attempts (Friedman test, $F_r = 12.22$, df = 3, p = 0.01; Figure 5) increased over the four trials. Additionally, their time to solve decreased over the four trials (Friedman test, $F_r = 25.56$, df = 3, $p < 0.001$; Figure 6). As we showed previously (Chapter 1), males' frequency of solving increased (Friedman test, $F_r = 22.24$, df = 3, $p < 0.001$; Figure 4), and time to solve decreased over the four trials (Friedman test, $F_r = 35.86$, df = 3, $p < 0.001$; Figure 6). However, males did not solve in fewer attempts over the four presentations of the barrier test (Friedman test, $F_r = 4.019$, df = 3, p = .26; Figure 5).

Female and Male Cognition

Males generally outperformed females on our barrier task. Males were more than twice as likely to solve on day one (Table 6, Figure 4), solved in 74% fewer attempts on day one (Table 6; Figure 5), and solved in 32% less time on day one (Table 6; Figure 6) than females. Males also improved in time to solve more than females did over the four presentations of the barrier test (Table 6, Figure 6). In contrast, males and females did not differ in learning performance as it relates to solving the barrier test (Table 6, Figure 4) or solves per attempt (Table 6, Figure 5).

Male Mate Choice

Male courtship behavior did not depend on female cognitive ability, regardless of the measure of cognition assessed (Table 7). Additionally, males courted females in better condition with greater vigor ($\chi^2 = 5.967$, df = 1, p = .015). Female condition had no impact on cognitive performance (Table 8).

Female Preference and Female Cognition

Female acceptance of males was not related to their cognitive performance with respect to the traits we measured. We found no significant interactions between female cognition and male sexual signals (Table 9). In some stickleback populations, acceptance of a male (entering their nests) is rare, therefore female acceptance is evaluated with other measures (Head et al. 2009; Kozak et al. 2009). However, in our population, females entered males' nests in 54% of trials, making acceptance a useful metric for this population.

Discussion

Our study is the first to examine the role of female cognition using an extractive foraging task (one unrelated to mate choice) on male and female mating decisions. We found dramatic differences in performance on the barrier test between the sexes, with males generally outperforming females. Males were more likely to solve the barrier test, solve it in fewer attempts, and solve it in less time on the first presentation (Table 6, Figures 4, 5, 6). Additionally, males were superior learners, reducing their time to solve at a greater rate than females over the course of the four presentations (Table 6, Figure 6).

Sex differences in cognition may reflect varied selection pressures. During the breeding season in other stickleback populations, males are solitary foragers, while females forage in shoals (Hart and Gill 1994). However, to our knowledge, no study has specifically looked at foraging behavior during the breeding season in SW Washington stickleback populations. Additionally, sticklebacks use social cues provided by conspecifics while foraging (Coolen et al. 2003). If females forage in groups, they may be more reliant on social cues, while males may be more reliant on knowledge gained

through personal experience. If foraging alone is more cognitively taxing or employs a different set of cognitive abilities, this may explain the sex-related differences in cognition that we found. Additionally, in some populations of sticklebacks, males have much larger brains compared to females (Kotrschal et al. 2012). These differences are likely due to males providing all the parental care, since in populations where males do not provide care this brain size dimorphism is reversed (Samuk et al. 2014). Parental care also covaries with brain size in cichlids, with the caring sex having larger brains (Gonzales-Voyer et al. 2009). Although our cognitive task did not directly assess parental care, cognitive ability has been shown to increase with brain size (Reader and Laland 2002), which may explain why males outperformed females in our cognitive task. Alternatively, our findings could be a product of differences in the stress levels of males and females (e.g. Harris et al. 2008). If foraging alone is more stressful for females, stress level may explain their poor performance.

Braithwaite and De Perera (2006) predicted that female sticklebacks might perform better on learning tasks because they commonly assess multiple male nests, then relocate high quality males to mate with them. While we did not directly test learning for the relocation of nests, we found that females either performed worse, or equivalent to males on our learning tasks, in contrast to this prediction. Males outperformed females with respect to learning on one aspect of performance: time to solve (Table 6). Quick decision-making and brief foraging bouts may be beneficial to males when they are defending territories relocating their nests, or raising/protecting offspring.

Male sticklebacks contribute extensively to parental care. Males should be choosy, more vigorously courting females who have traits that increase their fitness (Johnstone et al. 1996). We expected males would show a preference for females that performed well on our cognitive task. Although females from this population prefer males that problem-solve (Chapter 1), males did not express a significant preference for females that problem-solve (Table 7). Additionally, females in superior condition did not perform better than other females on our cognitive task (Table 8). This indicates that female condition is likely not a reliable signal for males to identify females with enhanced cognitive abilities. The asymmetry in preferences for cognition between the two sexes could again be a product of the sexes experiencing different selection pressures. During the breeding season, males select optimal nesting sites (Sargent 1982; Candolin and Voigt 1998), forage alone (Hart and Gill 1994), build a nest and provide all parental care (Wootton 1976), including supplying adequate oxygen (van Iersel 1953; von Hipple 2000) and defending against predators (Wootton 1976) and cannibalistic females (Whoriskey and FitzGerald 1985). Females, on the other hand, do not contribute to nest construction or parental care (Wootton 1976) and forage in shoals (Hart and Gill 1994). Females may prefer high performing males, because a male's contribution to future offspring depends greatly on his cognitive abilities. Our results demonstrate that males instead appear to prioritize female fecundity (courting females in better condition with greater vigor) over cognitive ability. However, unless these cognitive abilities are coded for on sex chromosomes, by ignoring female cognitive ability during mate choice, males run the risk of raising offspring with poor cognitive ability. We encourage more research

in this area. It will also be revealing to assess cognitive traits that may be more tightly linked to female fitness like the use of social information. Alternatively, because female sticklebacks lack flashy sexual signals, cognitive ability may be less easily communicated to mates, limiting males' ability to reliably identify high cognition females.

We found no evidence that high performing females choose different mates (Table 9). The complexity of mating displays and integration of information required to accurately assess them led us to hypothesize that female cognition may play a role in female mate assessment. Our cognitive test specifically tested individuals' ability to solve a novel foraging task, which may not translate well to the successful assessment of mating displays. For instance, social learning may be more important for female fitness and potentially more important when assessing other individual's traits. Alternatively, the assessment of elaborate signals may not be cognitively taxing.

In summary, we found that males consistently outperform females on a novel foraging task with respect to problem-solving measures and one learning metric. Although female sticklebacks consider male cognitive traits in their mating decisions (Chapter 1), male sticklebacks do not preferentially court females that perform well on the same cognitive tasks. These asymmetric interests may relate to differences in the cognitively taxing activities each sex carries out. Therefore, we do not expect cognition to be a unanimously preferred trait and preferences should instead depend on context. If males have mating preferences related to female cognition, male preferences may be for an entirely different suite of cognitive abilities that reflect cognitive challenges in females' lives.

Table 1. Effect of male color signals on performance on a novel barrier test. We consider three measures of cognitive performance on the first presentation of the barrier test (solving, solves/attempts and time to solve) and the same three measures over four presentations of the barrier test (learning). Male color signals predict high cognitive performance on some of our cognitive measures. Significant p-values are highlighted in bold.

Cognition Measures	**Fixed Effects**	χ^2	***df***	***P***
Solving Day 1	Body Mass	0.01	1	0.93
	Throat Score	2.43	1	0.12
	Eye Score	**6.59**	**1**	**0.01**
	Body Score	0.02	1	0.88
	Throat Score * Eye Score	**6.86**	**1**	**0.01**
	Throat Score * Body Score	**5.99**	**1**	**0.01**
Change in Solving (Learning)	Body Mass	0.17	1	0.69
	Throat Score	3.79	1	0.05
	Eye Score	**4.11**	**1**	**<0.05**
	Body Score	1.96	1	0.16
Solve/Attempts Day 1	Body Mass	0.08	1	0.78
	Throat Score	2.72	1	0.10
	Eye Score	1.39	1	0.24
	Body Score	1.20	1	0.27
Change in Solve/Attempts (Learning)	Body Mass	0.51	1	0.48
	Throat Score	0.21	1	0.65
	Eye Score	0.28	1	0.60
	Body Score	0.31	1	0.58
Time to Solve Day 1	Body Mass	**6.49**	**1**	**0.01**
	Throat Score	2.56	1	0.11
	Eye Score	0.33	1	0.57
	Body Score	3.44	1	0.06
Change in Time to Solve (Learning)	Body Mass	1.11	1	0.29
	Throat Score	0.02	1	0.89
	Eye Score	0.24	1	0.63
	Body Score	0.55	1	0.46

Table 2. Effect of nest area on performance on a novel barrier test. Again, we consider six measures of performance: solves, solves/attempts, and time to solve on day one of the barrier test, and solves, solves/attempts, and time to solve over the four presentations of the barrier test (learning). Males that constructed larger nests did not perform better on our cognitive measures.

Cognition Measure	χ^2	***df***	***P***
Solving Day 1	0.13	1	0.72
Change in Solving (Learning)	0.01	1	0.91
Solve/Attempts Day 1	>0.001	1	1.00
Change in Solve/Attempts (Learning)	0.02	1	0.90
Time to Solve Day 1	0.81	1	0.37
Change in Time to Solve (Learning)	0.02	1	0.88

Table 3: Effect of courtship vigor (number of male courtship behaviors per second) on performance on a novel barrier test. We consider six measures of performance: solves, solves/attempts, and time to solve on the first presentation of the barrier test, and solves, solves/attempts, and time to solve over the four presentations of the barrier test (learning). Males that courted with more vigor did not perform better on our cognitive measures.

Cognition Measure	χ^2	*df*	*P*
Solving Day 1	0.48	1	0.49
Change in Solving (Learning)	0.91	1	0.34
Solve/Attempts Day 1	0.90	1	0.34
Change in Solve/Attempts (Learning)	1.98	1	0.16
Time to Solve Day 1	1.37	1	0.24
Change in Time to Solve (Learning)	1.92	1	0.17

Table 4: Effects of male cognition on time to build a nest. Again, we consider six measures of performance: solves, solves/attempts, and time to solve on the first presentation of the barrier test, and solves, solves/attempts, and time to solve over the four presentations of the barrier test (learning). Males that performed better on our cognitive measures did not build nests quickly.

Cognition Measure	χ^2	*df*	*P*
Solving Day 1	0.06	1	0.81
Change in Solving (Learning)	0.02	1	0.89
Solve/Attempts Day 1	0.06	1	0.81
Change in Solve/Attempts (Learning)	0.11	1	0.74
Time to Solve Day 1	0.02	1	0.89
Change in Time to Solve (Learning)	0.07	1	0.80

Table 5: Effects of male cognition on female acceptance. We used generalized linear mixed models to examine the relationship between our six male cognition measures and female acceptance (entering a males nest). Male and female IDs were random effects and color signals (throat color score, eye intensity, and body intensity) and male body condition were covariates.

Cognition Measure	χ^2	***df***	***P***
Solving Day 1	**4.36**	**1**	**0.04**
Change in Solving (Learning)	0.01	1	0.93
Solve/Attempts Day 1	**4.62**	**1**	**0.03**
Change in Solve/Attempts (Learning)	1.40	1	0.24
Time to Solve Day 1	1.28	1	0.26
Change in Time to Solve (Learning)	<0.001	1	0.99

Table 6: Effect of sex (male vs. female) on performance on a novel barrier test. We consider three measures of cognitive performance on the day one of the barrier test (solving, solves/attempts and time to solve) and how the same three measures changed over four presentations of the barrier test (learning). Males outperformed females on the majority of cognitive measures tested. Significant p-values are highlighted in bold.

Cognition Measure	χ^2	***df***	***P***
Solving Day 1	**16.73**	**1**	**<0.001**
Change in Solving (Learning)	0.01	1	0.92
Solve/Attempts Day 1	**4.59**	**1**	**0.03**
Change in Solving/Attempts (Learning)	0.38	1	0.54
Time to Solve Day 1	**9.05**	**1**	**0.003**
Change in Time to Solve (Learning)	**4.77**	**1**	**0.03**

Table 7: Effect of female cognitive performance on male courtship vigor. We consider six measures of performance: solving, solves/attempts, and time to solve on the day one of the barrier test, and change in solving, solves/attempts, and time to solve over the four presentations of the barrier test (learning). Males courted females of varied cognitive abilities with equal vigor.

Cognition Measure	χ^2	***df***	***P***
Solving Day 1	2.30	1	0.13
Change in Solving (Learning)	0.87	1	0.35
Solve/Attempts Day 1	2.73	1	0.10
Change in Solving/Attempts (Learning)	0.24	1	0.63
Time to Solve Day 1	1.54	1	0.21
Change in Time to Solve (Learning)	1.13	1	0.29

Table 8: Effect of female condition on female cognitive performance. We consider six measures of performance: solving, solves/attempts, and time to solve on the day one of the barrier test, and change in solving, solves/attempts, and time to solve over the four presentations of the barrier test (learning). Females that were in better condition performed equally well on our cognition task.

Cognition Measure	χ^2	***df***	***P***
Solving Day 1	0.06	1	0.81
Change in Solving (Learning)	0.69	1	0.41
Solve/Attempts Day 1	0.03	1	0.84
Change in Solving/Attempts (Learning)	0.33	1	0.57
Time to Solve Day 1	0.84	1	0.36
Change in Time to Solve (Learning)	0.08	1	0.78

Table 9: Effects of female cognition on female mate acceptance. We used generalized linear mixed models to examine interactions between female cognition and male sexual signals on female acceptance. In each model we included one female cognition measure, a male signal (color or cognition), and the interaction of the two. A significant interaction between female cognition and male sexual signals or cognition indicates that female mating decisions depend on female cognition. We compared each model to a reduced null model that included the fixed effects but not the interaction to determine significance. We corrected for multiple comparisons by using a Bonferroni adjustment ($\alpha = .008$). We found no evidence that females of higher cognitive ability choose different mates.

Female Cognition Measure	Male Color Signal/ Cognitive Performance	χ^2	*df*	*P*
Solving Day 1	Throat Color	1.01	1	0.31
	Eye Color	1.17	1	0.28
	Solving Day 1	0.99	1	0.32
	Solves/Attempt Day 1	0.16	1	0.69
Learning to Solve	Throat Color	3.54	1	0.06
	Eye Color	2.32	1	0.13
	Solving Day 1	0.97	1	0.33
	Solves/Attempt Day 1	0.67	1	0.41
Solves/Attempts Day 1	Throat Color	0.03	1	0.87
	Eye Color	0.02	1	0.90
	Solving Day 1	0.88	1	0.35
	Solves/Attempt Day 1	0.34	1	0.56
Solves/Attempts Learning	Throat Color	0.05	1	0.83
	Eye Color	0.48	1	0.49
	Solving Day 1	0.09	1	0.77
	Solves/Attempt Day 1	1.37	1	0.24
Time to Solve Day 1	Throat Color	0.17	1	0.68
	Eye Color	0.17	1	0.68
	Solving Day 1	0.74	1	0.39
	Solves/Attempt Day 1	0.27	1	0.60
Time to Solve Learning	Throat Color	0.31	1	0.58
	Eye Color	2.01	1	0.16
	Solving Day 1	0.18	1	0.67
	Solves/Attempt Day 1	0.33	1	0.57

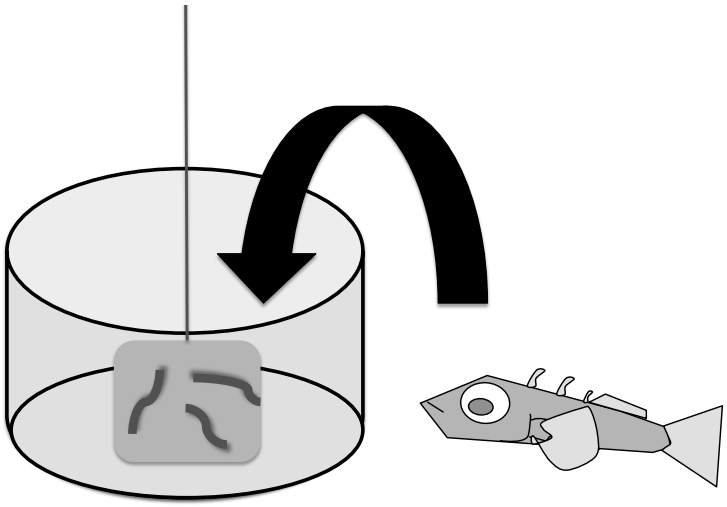

Figure 1: Barrier test apparatus used to measure cognition. Sticklebacks accessed the food reward (bloodworms on the outside of a clear bag, represented by dark grey lines) by swimming above and into the cut out circle on the top of the clear, cylindrical barrier.

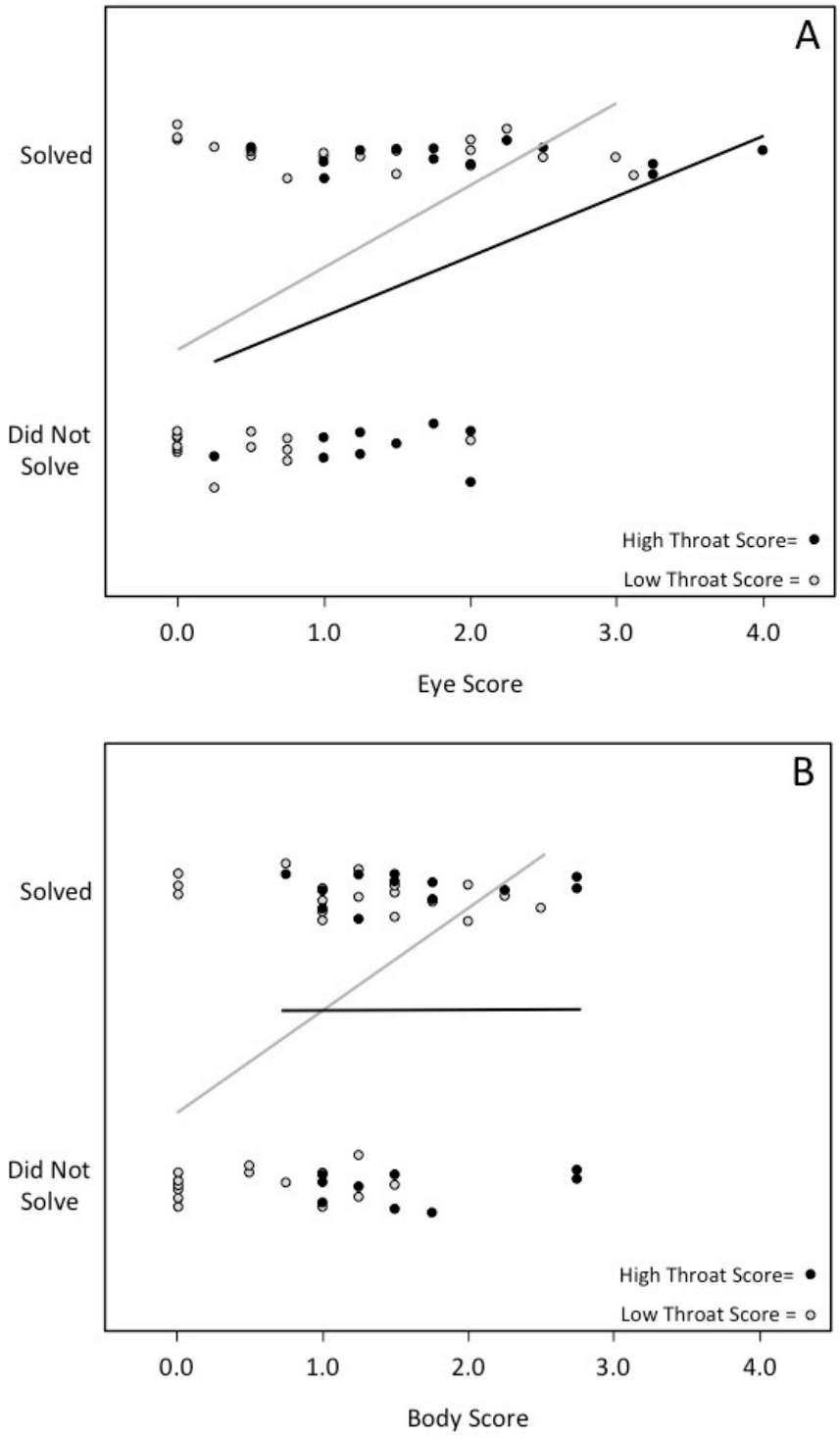

Figure 2: (a) The interaction of male eye color score and throat color score on solving the barrier test on day one (a measure of problem solving) ($p = 0.01$). (b) The interaction of male body color score and throat color score on problem solving ($p = 0.01$).

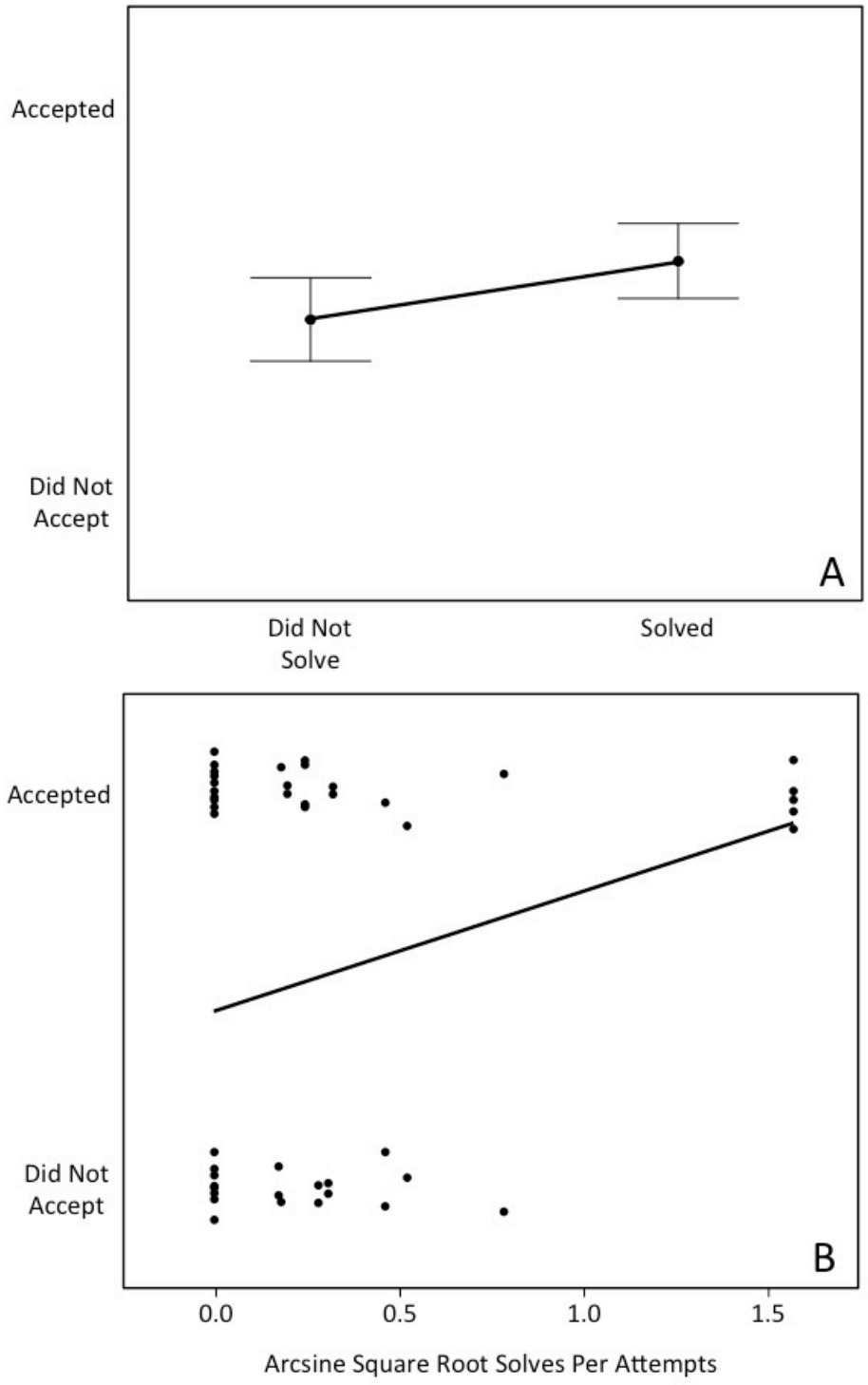

Figure 3: (a) Females were more likely to accept (entering the nest) males that problem solve ($p = 0.04$). (b) Females were more likely to accept (entering the nest) males that solved the barrier in fewer attempts during first presentation ($p = 0.03$).

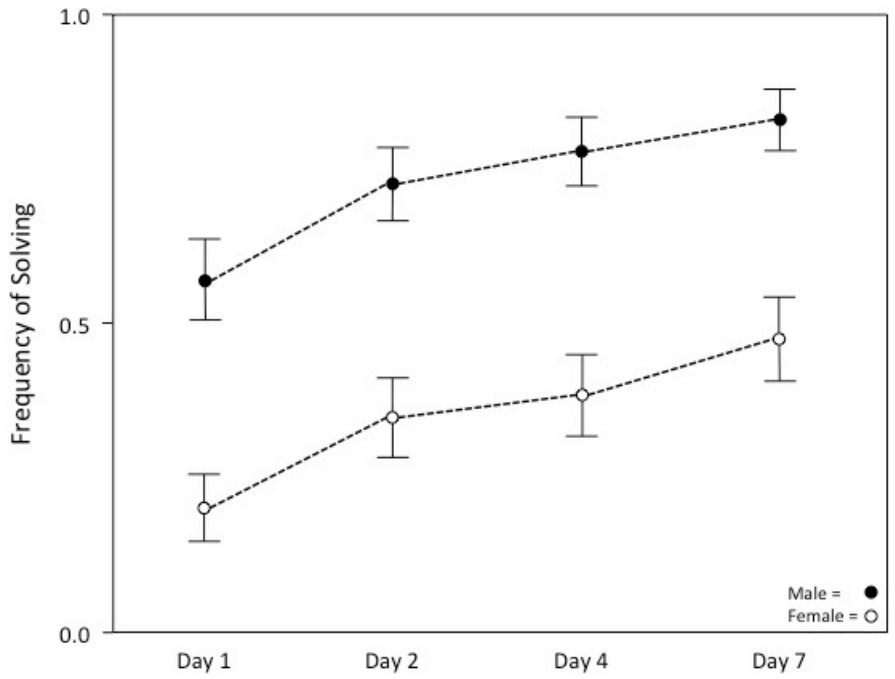

Figure 4: Males ($p < 0.001$) and females ($p < 0.001$) both increased in solving frequency over time. Additionally, males were more likely to innovate (solve the barrier test on day one; $p < 0.001$) than females. However, the slope of the male and female learning to solve curves did not differ ($p = 0.92$).

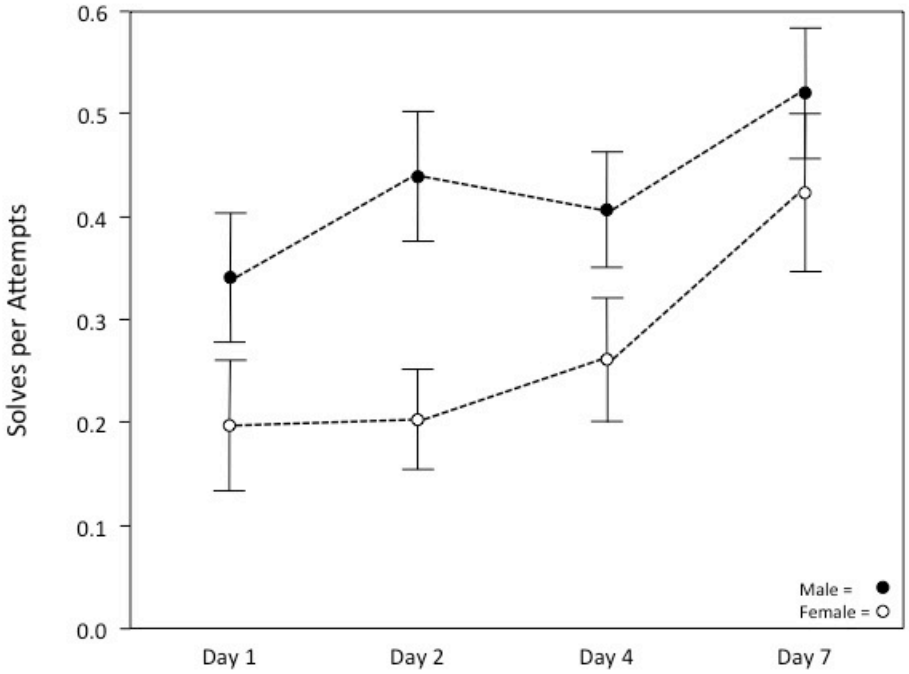

Figure 5: Females solved the barrier test in fewer attempts over time ($p = 0.01$), but males did not ($p = 0.26$). Additionally, males were more likely to solve in fewer attempts on day one ($p = 0.03$) than females. However, male and female learning curves did not differ for solves per attempt ($p = 0.54$).

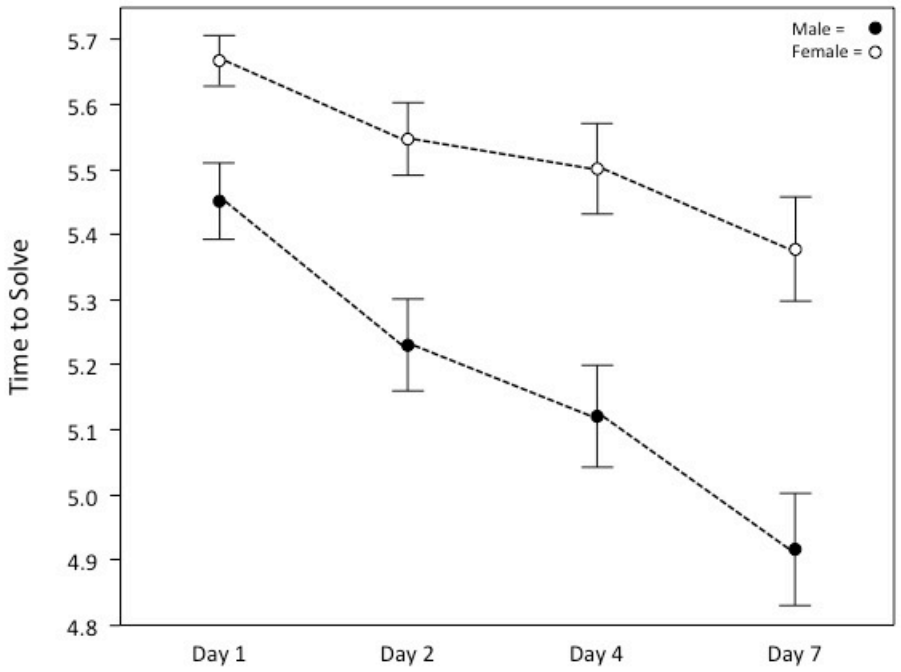

Figure 6: Males ($p < 0.001$) and females ($p < 0.001$) solved the barrier test in less time over the four presentations of the test. Additionally, males solved more quickly on trial one ($p = 0.003$) and deceased in solving time over the four trials at a faster rate than females (steeper learning curves for time to solve) ($p = 0.03$).

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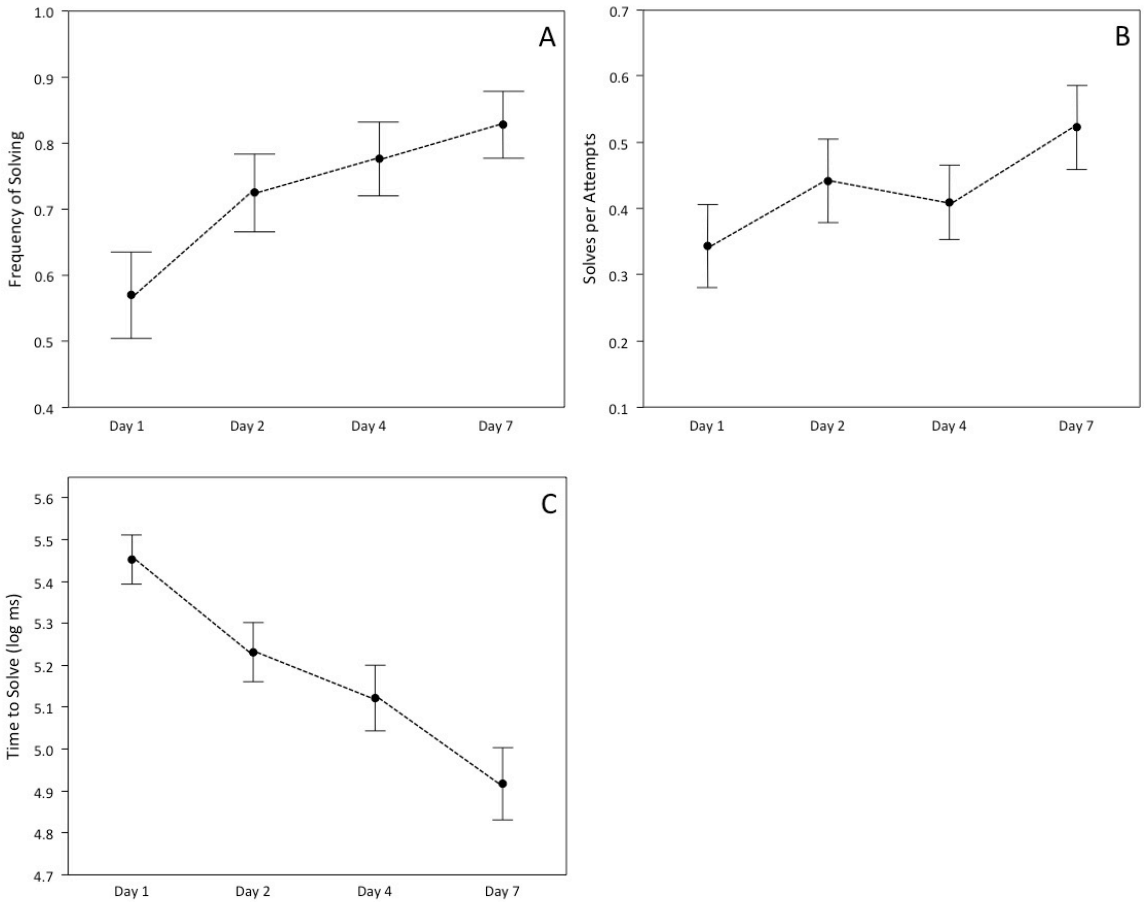
APPENDICES

Appendix 1 Male and female courtship behaviors recorded using JWatcher during no-choice courtship trials.

Male Courtship Behaviors	Female Courtship Behaviors
Male Approach: male movement towards female to within 2 cm	**Angle**: female's body at 45° incline
Bites: male nips female with mouth	**Approach**: female movement towards male to within 2cm
Chase: male swims behind female vigorously	**Enter Nest**: female goes into the males nest
Dorsal Pricks: male sticks female with dorsal spines	**Examine**: female inspects the males nest
Fanning Nest: males uses his pectoral fins to oxygenate his nest	**Follow**: female trails male after a "Lead"
Lead: male is near female, turns away and guides her to nest	**Hover**: female positions herself above male
Nesting: male is constructing his nest	**Head-up**: swift motion into an "Angle", requires active effort
Show Nest: male displays the entrance of the nest	**Swimming**: female moves forward through the water at least half a body length
Through Nest: male goes through nest entirely while "Nesting"	
Zig Zag: quick left-right movements in a zigzag pattern	



Appendix 2 (a) The frequency (%) of males solving increased over the four trials ($p < 0.001$). (b) Males did not change in solves per attempt on the barrier test over the four trials ($p = 0.25$). (c) Males solved the barrier test in less time over the four trials ($p < 0.001$).